

The Insoluble Carbohydrates of Wheat

(TRITICUM VULGARE)

—BY—

HENRY CLAPP SHERMAN

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN

THE FACULTY OF PURE SCIENCE,

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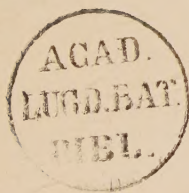
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INTRODUCTION.

The work recorded here originated in a consideration of methods for the quantitative separation of the carbohydrates in cereals and their products.

A somewhat extended study of the determination of starch gave very satisfactory results.

In the case of other insoluble carbohydrates, however, it was found necessary to know more of their nature before attempting their separation. Wheat was selected for this study. Quantitative methods have been employed wherever possible and it is hoped that the results obtained may be of interest in themselves and of use as a basis of analytical operations.

The determinations of digestibility serve the double purpose of giving a suggestion as to the value of these different carbohydrates and of illustrating the possibility and the importance of their separation. For the samples used in this part of the work, I am indebted to Prof. H. J. Patterson.

My thanks are due to Prof. Charles F. Chandler, of this University, with whose approval this work was undertaken, and to Prof. Ricketts and Dr. Vulté, of the analytical laboratory, under whose supervision it was performed.

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PART I—DESCRIPTIVE.

A. WHEAT STARCH.

The constitution of wheat starch has been thoroughly investigated by L. Schulze.¹ A very carefully prepared air-dried starch was employed, allowance being made in the calculations for moisture present in the sample. His results, obtained both by elementary analysis and by estimation of the dextrose formed on hydrolysis, show conclusively that the formula of wheat starch is $n(\text{C}_6\text{H}_{10}\text{O}_5)$ and not, as supposed by Naegeli, $\text{C}_{36}\text{H}_{62}\text{O}_{31}$.

The separation of starch from accompanying carbohydrates has been discussed in another paper² and will be referred to in speaking of analytical methods (page 17).

B. HEMICELLULOSE—PENTOSANS.

The term hemicellulose has been introduced by E. Schulze³ to designate the carbohydrate matter yielded to boiling dilute acids by vegetable cells from which the starch has been removed. It is a portion of the cell-wall rather than of the cell contents.⁴ The fact that the hemicellulose of wheat yields both arabinose and xylose on hydrolysis has already been noted.⁵ In general, however, it must be said that the amount of substance which was obtained for identification was so small in proportion to that hydrolyzed as to suggest the question whether the result could be taken as proving the absence of other sugars. I decided to try a method which should not depend upon recrystallization.

Preparation of Sample.—Wheat bran⁶ was extracted successively with water, saline solution, malt extract, two per cent. ammonia, cold two per cent., and boiling one-tenth per cent. sodium hydroxide. Each reagent was removed by thorough washing with water before the application of the succeeding one. This treatment removed, beside fatty and resinous matter, all of the soluble carbohydrates and starch and nearly all of the proteid

¹ *J. prakt. Chem.*, **136**, 311.

² *School of Mines Quarterly*, **17**, 356.

³ *Ztschr. phys. Chem.*, **16**, 387-391.

⁴ Prof. C. Cramer, in connection with Schulze's work.

⁵ Stone: *Ann. Chem. (Liebig)*, **249**, 239; Steiger and Schulze: *Ber. d. chem. Ges.*, **23**, 3110; E. Schulze: *Ztschr. phys. Chem.*, **16**, 397.

⁶ Bran was used instead of the whole grain because it contains all the insoluble carbohydrates of the latter and does not require the removal of so much starch.

matter without the use of any acid. The residue consists essentially of cell-walls.¹

The residue from the extraction with ammonia contained, in the air-dried state, 8.30 per cent. moisture, 8.50 per cent. proteids (nitrogen $\times 6.25$), and 3.69 per cent. ash. It yielded, on the basis of non-nitrogenous ash-free substance, 27.62 per cent. furfural (determined as described in Part II) equivalent to 50.82 per cent. pentosans.

The sodium hydroxide extract contained pentosans, giving the characteristic red coloration with phloroglucin in hydrochloric acid and yielding furfural when distilled with hydrochloric acid.

The air-dried residue contained 7.65 per cent. moisture, 1.25 per cent. proteids, and 3.31 per cent. ash. On the basis of non-nitrogenous, ash-free substance, it yielded 25.62 per cent. furfural equivalent to 48.13 per cent. pentosans.

Hydrolysis of the Hemicellulose.—As it was necessary to choose a somewhat arbitrary treatment, I adopted that which forms a part of the ordinary method of food analysis; *viz.*, thirty minutes boiling with 1.25 per cent. sulphuric acid. About one-half of the substance dissolved. The residue was washed free from acid and preserved for further examination.

To the solution sufficient sulphuric acid was added to bring the total present to two per cent., and it was then boiled gently with a reflux condenser until the reducing power of the solution no longer increased (six hours). This reducing power was found to be equivalent to 91.2 per cent. of that of dextrose and showed that the solids in solution consisted almost entirely of reducing sugars. A second determination showed the organic solids in solution to have a reducing power of 91.3 per cent. of that of dextrose.

Qualitative Tests.—The solution was tested² for mannose with phenylhydrazine acetate, for galactose by evaporation with nitric acid, and for levulose by resorcin in hydrochloric acid. All of these tests gave negative results. Reactions of the pentoses indicated their presence in large proportion. No direct test

¹ Cramer: *Loc. cit.*

² Lippmann: *Chemie der Zuckerarten*, 338, 395, and 483.

for dextrose, applicable under these circumstances being known, the osazone was next prepared.

Preparation of the Osazone.—This was carried out essentially as described by Gans and Tollens.¹ A part of the solution was concentrated on the water-bath until it contained ten per cent. of solids, cooled to 70°, and then mixed with an equal volume of a solution containing for each gram of carbohydrate treated, two grams phenylhydrazine hydrochloride and three grams of sodium acetate. The mixture was kept at 65°–70° for about forty-five minutes and then allowed to cool. The osazone separated as a yellow iridescent precipitate which was collected, washed thoroughly and dried without recrystallization. Thus it was hoped to secure a product nearly representative of the entire solution, and whose composition should show the relative proportions of pentose and hexose present. As the yield of osazone is never quantitative, this method cannot be regarded as absolutely accurate, but, on the other hand, both dextrose and the pentoses form their osazones easily and freely under the conditions described.

Since Stone² has found that the osazones of the pentoses are soluble in about fifty parts of water on continued boiling, while glucosazone is quite insoluble, the proportion of the latter, if any were present, could be largely increased by dissolving away most of the pentose compound. Accordingly a part of the osazone described above was boiled for ten minutes with about forty parts water, whereby about three-fourths was dissolved. The residue was dried and analyzed as was the original preparation.

Analysis of the Osazones.—The percentage of nitrogen in each of the preparations was determined by Dumas' method with the following results :

First Preparation. — Original precipitate; *a.* 0.1716 gram yielded 26.6 cc. nitrogen at 29.5° and 764.4 mm. pressure.

b. 0.1694 gram yielded 25.8 cc. nitrogen at 20.0° and 759.4 mm. pressure.

Second Preparation.—Residue; 0.0993 gram yielded 14.8 cc. nitrogen at 21.0° and 761.0 mm. pressure.

From which we obtain :

¹ *Ann. Chem.* (Liebig), 149, 249.

² *Am. Chem. J.*, 15, 662.

	Nitrogen. per cent.	Nitrogen. per cent.
Original precipitate	{ a. 16.98 b. 17.16 }	Average.. 17.07
Residue from boiling water		16.99
Calculated for $C_{17}H_{20}N_4O_3$ (from pentose)		17.07
“ “ $C_{18}H_{22}N_4O_4$ (from hexose)		15.64

The hemicellulose of wheat appears then to yield on inversion only pentoses and, therefore, to consist of pentose anhydrides or true pentosans.

C. CHARACTERISTICS OF THE FIBER.

Composition and Color Reactions.—The residue from the action of sulphuric acid, after washing with water and alcohol and drying in the air, was of a medium brown color and loose fibrous texture. It contained 3.43 per cent. moisture, 0.65 per cent. ash, 0.25 per cent. nitrogen, and yielded 11.46 per cent. furfural. It showed well the deep magenta color (produced by lignin) when treated with chlorine and then boiled with sodium sulphite, and gave the red coloration when heated with the phloroglucin reagent (test for pentosans). No coloration was obtained on treating the fiber with a solution of fuchsin decolorized with sulphurous acid nor on boiling with anilin sulphate (tests for oxycellulose).

Reactions with Ferric Chloride and Potassium Ferricyanide.—Jute fiber, when immersed in a solution containing molecular proportions of the above reagents, fixes upon itself a large proportion of ferrous ferricyanide as a uniform deep-blue dye. This is considered by Cross and Bevan¹ as one of the most characteristic reactions of the lignocelluloses of which jute is the type.

In my experiments on the wheat fiber, I used a fresh mixture of equal volumes of aqueous solutions containing in 100 cc., one and six-tenths grams ferric chloride and three and three-tenths grams potassium ferricyanide respectively. The increase in weight of the fiber after immersion in this solution was determined by collecting in a Gooch crucible, washing well with cold water, and drying at 105°. The following quantitative experiments were made:

¹ Cellulose, p. 124-131.

SERIES A.—TIME VARIED. MASSES CONSTANT.

Weight of fiber.	Volume of mixed solution.	Time of immersion.	Increase in weight.
Gram.	cc.		Per cent.
1.00	20	20 min.	9.40
1.00	20	1 hour	11.17
1.00	20	10 hours	11.16

With these proportions, then, the reaction proceeds rapidly to the maximum.

SERIES B.—TIME CONSTANT. MASSES VARIED.

Weight of fiber.	Volume of mixed solution.	Time of immersion.	Increase in weight.
Gram.	cc.	Hours.	Per cent.
1.00	20	16	11.26
1.00	40	16	21.81
1.00	60	16	30.49
1.00	80	16	36.71
1.00	100	16	46.70

Wheat fiber has, therefore, like the typical lignocellulose (jute), the power of fixing upon itself an amount of the cyanide varying roughly with the amount exposed to its action. This reaction is very interesting as showing the similarity of the lignocellulose molecule in these tissues. It will be shown to be of value also as a test of the purity of cellulose.

D. LIGNONE CHLORIDE.

Cross and Bevan¹ have isolated a compound from chlorinated jute fiber to which they give the above name. Its composition corresponds to the formula $C_{19}H_{18}Cl_4O_9$ and is constant after fractional precipitation and when prepared under varying circumstances. It is precipitated as yellow flocks when its alcoholic solution is diluted with water.

Seventy-five grams of the fiber already described was boiled with one per cent. sodium hydroxide, washed, pressed to remove most of the water, treated with chlorine gas for one hour, washed free from hydrochloric acid, and covered with alcohol. On pressing out the alcoholic solution it was found to be of a deep golden yellow color. On concentrating and pouring into water a part only of the dissolved substance separated, as was shown by the color of the supernatant liquid. The precipitate obtained, although small (about one-half gram), corresponded in appearance with that described and on collection

¹ Cellulose, p. 135.

and analysis showed 26.7 per cent. chlorine, or just the amount required by the above formula. This seems sufficient evidence of the identity of the products.

I do not know that this compound has previously been prepared from any other source than commercial fibers, but the fact that it may be obtained from such a widely different tissue as that of the coat of the wheat kernel would indicate that this type of lignification may be general among plants of annual growth.

On the other hand, the amount recovered is too small to justify much speculation until further results are obtained.

E. CELLULOSE.

For the separation of cellulose three very different methods were tried.

1. *F. Schulze's Method.*—Thirty grams of the fiber was treated at ordinary temperature with a solution of twenty-five grams potassium chlorate in 350 cc. of nitric acid (1.10 sp. gr.). After standing seven days with occasional stirring, the strength of nitric acid was raised to 1.13 sp. gr. by the addition of concentrated acid, and the digestion continued for another seven days. Finally, the mixture was kept at about 40° for two hours, then filtered, washed free from acid, and treated on the filter with cold two per cent. ammonia as long as the filtrate was colored, and the washing finished with water and alcohol.

The sample seemed particularly resistant to this treatment so far as appearance was concerned. Instead of becoming white, it attained during the first two or three days a dull flesh color which was not materially changed during the remainder of the fortnight. The color of the dried residue was light brown, very much lighter than, but still suggesting, that of the original fiber. By repeating the treatment or increasing its severity a purer residue could doubtless be obtained but, as the yield is already less than that from the method next to be described, there seems no object in experimenting in this direction.

The residue constituted 66.0 per cent. of the original. It yielded 7.00 per cent. furfural and gave the red coloration when heated with phloroglucin. When boiled with anilin chloride it was tinged very faintly reddish, indicating a trace of oxycellulose. From twenty parts of the standard cyanide solution it

absorbed 6.04 per cent. of its weight of the pigment, indicating, as comparison with the other cellulose will show, that the lignin group was not selectively attacked.

2. *Method of Cross and Bevan.*—Thirty grams fiber was boiled thirty minutes with 800 cc. of one per cent. sodium hydroxide, filtered, washed with water till free from alkali, squeezed as free from the water as possible, and then exposed in this moist condition to the action of chlorine gas in a covered beaker at ordinary temperature for one hour with occasional stirring. The color changed rapidly from brown to light lemon-yellow. At the end of the hour it was brought upon a filter and washed with water till free acid was removed, then heated to boiling with 600 cc. of two per cent. sodium sulphite and sufficient sodium hydroxide solution added to make two-tenths per cent. of the whole. The boiling was continued five minutes, the solution filtered hot and the residue washed until the washings were neutral and colorless. It is convenient to use a muslin filter and hasten the operation by squeezing out the washings. Finally the washing was finished with alcohol and the residue dried and weighed.

This process yielded 66.5 per cent. of residue of a light creamy color and free from nitrogen. It yielded 5.62 per cent. of furfural and gave a distinct coloration with phloroglucin, but not with anilin chloride. From the cyanide solution it absorbed only 0.92 per cent.

3. *Lange's Method of Fusion with Alkali.*—Bring five to ten grams of the (fat-free) substance into a porcelain crucible about three inches high with three times as much caustic potash and about twenty cc. of water. Heat with stirring in an oil-bath not above 180° , keep at this temperature (175° – 180°) for an hour, cool to 75° – 80° , add about seventy-five cc. hot water, cool, acidulate with sulphuric acid, make slightly alkaline with sodium hydroxide, and separate the precipitate by means of a centrifugal machine. The liquid is poured off and the cellulose washed with hot water, separating in the same way. Bring onto a filter; wash with water, alcohol and ether, dry and weigh. Incinerate and deduct the ash.¹

Ten grams were used for each determination; the melting pro-

¹ *Ztschr. angew. Chem.*, 1895, 561.

ceeded quietly and without troublesome frothing. The precipitate was allowed to settle by standing instead of separating by centrifugal force. Thus obtained it is of a somewhat slimy character, and the process of filtering and washing is therefore slow. This is the only difficulty of manipulation which the method presents. The cellulose dried to a white powder, the fibrous nature having been destroyed by the treatment.

Lange obtained higher results by this method than by that of Schulze, but in this case I recovered only 39.3 to 43.1 per cent. cellulose (without allowing for ash). The product yielded 3.96 per cent. furfural and gave the phloroglucin reaction, although not so markedly as did the other preparations of cellulose. Boiled with a solution of anilin salt it showed no evidence of any oxycellulose. From the cyanide solution it absorbed 0.89 per cent.

Choice of Method of Preparation.—For reasons which are mainly obvious, but which will be reviewed in speaking of analytical methods, the process of separation by means of chlorination is preferred, and cellulose thus obtained was employed in the following experiments.

Action of Strong Sulphuric Acid.—Strong sulphuric acid (100 parts sulphuric acid and seventeen parts water) dissolves pure cotton cellulose with only very slight coloration and the solution when diluted and boiled yields dextrose. E. Schulze¹ has found the same to take place with cellulose residues from other natural tissues prepared by vigorous acid hydrolysis followed by F. Schulze's reagent, although the amounts of dextrose obtained were small.

When applied to the cellulose described above, under the conditions given by Schulze, the solution was not complete and a very considerable discoloration was produced. The undissolved portion (nearly one-fifth) did not yield a measureable amount of furfural. The final product of hydrolysis of ten grams of the cellulose, converted into osazone in the usual way, gave only 1.31 grams of the latter. Its composition is shown by the following results:

a. 0.1914 gram gave 25.9 cc. nitrogen at 23.8° and 775.5 mm. pressure, equal to 15.48 per cent. ; *b.* 0.1870 gram gave 24.7 cc.

¹ *Ztschr. phys. Chem.*, 16, 414.

nitrogen at 17.0° and 771.0 mm. pressure, equal to 15.58 per cent.; average 15.53 per cent.; calculated for $C_{18}H_{22}N_4O_4$ 15.64 per cent.

The osazone is, therefore, that of dextrose and the specific products of hydrolysis of the furfural-yielding group do not appear.

Action of Cold Dilute Sodium Hydroxide.—Twenty grams cellulose was treated with 400 cc. five per cent. sodium hydroxide at room temperature with occasional stirring for two days, then filtered and washed. The filtrate was light brown in color, and on neutralizing with hydrochloric acid a white precipitate was formed. I was not able to separate this precipitate quantitatively, but enough was collected to show its general resemblance to wood gum, and it gave the characteristic deep red coloration with phloroglucin. The residue constituted about eighty per cent. of the amount treated. It was whiter than before treatment. A determination of furfural gave 3.34 per cent., equal to 2.67 per cent. of the original. Hence less than one-third of the dissolved substance was pentosan, and the suggestion of Hoffmeister¹ that this treatment be inserted as a step in the determination of cellulose, would seem of no value.

Chemical Nature of the Cellulose.—The term cellulose is here used in an inclusive sense to designate the strictly carbohydrate radicle as opposed to the more or less "condensed" lignin substance with which it is combined in the lignocellulose. The abnormal characteristics of this sample (property of yielding furfural and coloring phoroglucin reagent) I believe to be due to the inherent nature of the cellulose itself rather than to imperfect methods for its separation, except in the case of Schulze's method. The sample contains about ten per cent. of penta-anhydride which appears to be chemically combined with a part at least of the hexa-anhydrides (normal cellulose). It may be well now to review some of the reasons for this belief.

1. The absence of lignin groups is strongly indicated (*a*) by the fact that the repetition of the chlorination followed by treatment with sodium sulphite gave no red coloration, and very slight loss of weight, (*b*) the insignificant amount of cyanide absorbed (less than one per cent.)

¹ Landw. Versuch-sta., 39, 461.

2. The method of fusion with alkali, totally different in its nature, causing complete disintegration of the fiber and a much lower yield than the chlorination method, still produces a cellulose in which the abnormal characters differ only in degree from those shown by the product of the latter process.

3. The imperfect solubility in sulphuric acid and the small amount of glucosazone formed from the product of hydrolysis indicate that the cellulose is not of the normal type.

4. The proportions of substances extracted by dilute alkali show that the sample is not a simple mixture of pentosan and normal cellulose.

5. Somewhat similar characters have been found by others¹ in studying natural celluloses prepared by other methods than those (Cross's and Lange's methods) to whose products the above statements refer.

F. FURFURAL-YIELDING CONSTITUENTS.

Some of the facts observed in connection with this class of bodies would seem worthy of mention here. They constitute nearly or quite one-half of the seed-coat exclusive of starch and proteids—a much higher proportion than is found in woods, straws, or commercial fibers—and by far the greater part is in the form of easily hydrolyzed pentosans. The physical effect of these in rendering the tissue more tenacious and more retentive of moisture is doubtless of considerable physiological significance. It was observed in the course of this work that the samples which had not been treated with acid held about twice as much hygroscopic moisture as did those from which the pentosans had been removed, when similarly air-dried. In the case of isolated xylan, this affinity for moisture is so great as to be a most serious obstacle to its analysis.²

The wide distribution of furfural-yielding bodies among the different chemical groups which compose the cell-wall has been shown by the quantitative determinations mentioned above, in the original tissue, in the fiber remaining from acid treatment, in the cellulose isolated from the latter by various methods, and

¹ C. Schulze and Tollens: *Landw. Versuch-sta.*, 40, 367; Hoffmeister: *Landw. Versuch-sta.*, 39, 461; Winterstein: *Ztschr. phys. Chem.*, 17, 391; E. Schulze: *Ztschr. phys. Chem.*, 16, 414.

² Johnson: *J. Am. Chem. Soc.*, 18, 214.

in cellulose which had been further treated with dilute sodium hydroxide. We have also seen that all of these specimens gave distinct pentosan reactions¹ with phloroglucin and that none of them gave distinct oxycellulose reactions with anilin salts. We therefore conclude that the furfural-yielding constituents of wheat are essentially pentosans in some form and that we commit no serious error in interpreting the amount of furfural obtained as a measure of the total pentosans present. But if we should use the term *pentosans* in this inclusive sense and stop here, it would be little more satisfactory than to include dextrin, starch, and cellulose in one group of *hexosans*. Our pentosans would constitute practically all of the hemicellulose, about forty per cent. of the lignin group or non-cellulose of the fiber, and about ten per cent. of the cellulose (chlorination method). It would seem much better, therefore, to distinguish between those pentosans which are easily dissolved by dilute acids without materially affecting the cellulose or lignin, and those which on the other hand, are resistant to acid, or to both acid and alkaline, hydrolysis. The former are evidently not combined with hexose groups and they may be considered as true or free pentosans; the latter are apparently in chemical combination, partly in the lignin group and partly in the cellulose.

¹ While the work was in progress the results of Cross and Bevan on the furfuroids of straws appeared (*J. Chem. Soc.*, 69, 804-818). In view of the purple reactions with phloroglucin found by them, it was thought best to retest all the celluloses side by side with a pentose solution. Especially in the cases where the reaction was least marked, the color appeared somewhat darker and not so clear as in the pentose solution, but the difference seems to be due to the degree of heat used to develop the color. When the phloroglucin compound was precipitated by boiling and then dissolved in alcohol, no such differences were found in the alcoholic solutions.

SCHEME OF ANALYSIS USED.¹

Five grams of the sample (previously extracted with ether) are stirred with about 100 cc. water for a few minutes, filtered, and washed.			
Filtrate. —Add one-tenth its volume of 25 per cent. HCl and heat with a reflux condenser for two and one-half hours on a water-bath, or boil thirty minutes on a sand-bath. Clarify, if necessary, and determine the dextrose present in an aliquot portion, by means of Fehling's solution, and calculate to	Residue. —Wash into a beaker with about 100 cc. water (more if the proportion of starch is high), heat to boiling, cool partially, and treat with malt extract till starch disappears, filter, and wash.		
	Filtrate. —Treat as in the case of the preceding filtrate and calculate the dextrose found to	Residue. —Boil thirty minutes with 1.25 per cent. H_2SO_4 , filter, and wash with water and alcohol.	
		Filtrate. —Add sufficient H_2SO_4 to make 2 per cent. Boil gently with reflux condenser for six hours. Determine reducing power; calculate to pentose and thence to	Residue. —Dry and weigh. In a duplicate residue, determine proteid (and ash) and correct the weight accordingly. Treat according to the method of Cross and Bevan, dry, and weigh the residue.
		Loss of weight corrected for proteids (and ash) present (and ash), equals	Residue, less proteid if present (and ash), equals
Soluble carbohydrates as dextrin.	Starch.	Free pentosans.	Lignin and allied substances.
			Cellulose.

¹ Since this work was finished, a method for the determination of carbohydrates in cereals, devised by Prof. W. E. Stone, has been published by the U. S. Department of Agriculture (Bul. 34, Office of Experiment Stations). See also *J. Am. Chem. Soc.*, 19, 183 and 349.

PART II—ANALYTICAL.

A. SEPARATION OF THE INSOLUBLE CARBOHYDRATES.

It is customary in cereal analysis either to be content with the results of the Weende method or to determine in addition the starch and perhaps also the furfural obtainable and calculate the latter to pentosan. The latter determination we have seen to be to a certain extent misleading since a part of the furfural comes from bodies in combination both in the cellulose and in the lignin groups and while apparently of a pentose nature, these are quite different in their properties from the true pentosans of the hemi-cellulose. In order to separate quantitatively the carbohydrates described in the first part of this paper, it is necessary to combine the methods best adapted to the determination of each into a single scheme. So much time was required to test the different methods and the products yielded by them that some of the details are in need of further study and the results are offered as approximate rather than absolute or final. An outline of the scheme is given and is followed by a discussion of the different steps. An application of the scheme is found in Part III.

I. THE DETERMINATION OF STARCH.¹

Many methods have been proposed for the determination of starch. A list of the principal ones is appended to this paper. An examination of these methods shows that, while most of those pretending to general application are based on the hydrolysis of the starch and estimation of the resulting glucose, they differ widely in method of treatment.

The fact that most of these methods give high results when applied to materials containing much other insoluble matter has been most clearly recognized and understood since the researches of Tollens, Stone, and others, have revealed the wide distribution and susceptibility to hydrolizing agents of the pentosans, xylan, and araban bodies bearing the same relation to the pentose sugars which starch bears to glucose. Of these xylan is most characteristic of grains and grasses.² Experiments on isolated xylan, and on natural products containing the same, show that it is affected by most acids almost as readily as starch, and

¹ Reprinted from the *School of Mines Quarterly*, [4], Vol. XVII.

² Stone ; Bull. 43, U. S. Dept. Agr., Div. Chem., p. 165.

more readily than any of the other insoluble carbohydrates which have been isolated from grains and studied.

Many important researches on food materials have been based on determinations of starch by methods devised before the isolation of the pentosans, and hence without a knowledge of their properties.

It seemed, therefore, of interest and importance to compare some of the methods most commonly used in the past with those now considered most promising, not only to determine how far the former may be applied with safety, but to indicate to what extent the results formerly obtained can be accepted as correct. Whenever possible, the accuracy of the different methods has been tested directly, and for the reasons given above, special attention has been directed to the pentosans.

The property of yielding furfural on distillation with acids is usually considered as characteristic of the pentose carbohydrates and is possessed both by the natural substances and the sugars derived from them.¹ This furnishes a means of testing the solutions which we obtain in determining starch, to discover whether pentoses are present. A positive result shows at once that, other conditions being correct, our results will be too high from the reduction of Fehling's solution by the pentose. A negative test proves that no error is introduced from this cause. The manner in which I have applied this test is as follows:

Bring about twenty-five cc. of the solution to be tested into a small lipped beaker, add the same volume of hydrochloric acid twenty-five per cent. This gives the acidity most advantageous to the production of furfural. Cover with a watch glass and heat to boiling. When the mixture boils briskly, introduce a slip of filter paper moistened with anilin acetate (made by mixing equal volumes of anilin and strong acetic acid) into the escaping vapors. If furfural is given off, a bright red coloration appears. A comparison of Tables I and II below shows a close relation between the results of these tests and the percentages indicated by the different methods.

Materials Employed.

The pure starch sample used was prepared by repeatedly

¹ Since oxycellulose, in the natural state, is less readily attacked than the pentosans, it may for our present purpose be disregarded.

washing good corn starch with dilute saline solution and water, and finally drying the purified product in the air. Careful determinations in duplicate showed: ash, 0.20 per cent.; moisture, 17.36 per cent.; starch, by difference, 82.44 per cent. The other samples were commercial products of apparently average quality. All were ground to pass the 60-mesh sieve. In every case the water-soluble matter was extracted before proceeding with the analysis. Failure to do this caused, in the case of the grains, errors of from two to five per cent. Previous extraction with ether was tried on the wheat flour and oat-meal but caused no difference in the results.

Inversion on the Sand Bath.

Hibbard¹ has proposed thirty minutes actual boiling on the sand bath, in lieu of two to two and one-half hours heating on the water bath, to effect the final inversion of the products of the action of diastase on starch. His results (volumetric) were identical by the two methods. I have made the following experiment: Equal portions of such a solution were treated with the same amounts of acid and inverted by the different methods, all the other conditions being exactly the same and the reducing power of the final solutions being estimated by boiling twenty-five cc. exactly two minutes with excess of Fehling's solution. Results:

Inverted on sand bath. Practically colorless.

Milligrams copper reduced.	
	194.5
	194.4
	195.5
Average	194.8

Inverted on water bath. Very faint straw tint.

Milligrams copper reduced.	
	194.4
	194.3
	194.5
Average	194.4

In similar cases in the following work I have inverted in this

¹*J. Am. Chem. Soc.*, 17, 64.

manner and have had no reason to doubt the accuracy of the results.

Description of Methods.

The inversion methods have always been the ones principally used. These may be divided into four classes, according to the degree to which the inversion is carried before the removal of foreign matter from the influence of the solution, as follows: (a) complete hydrolysis to glucose; (b) conversion to maltose and dextrin by ferments; (c) conversion to dextrins by acids; (d) solution without chemical change. The methods which have been studied will be taken up according to this arrangement. For references see the appended list. Results are given in Table I.

(a) *Sachsse's Method*.—The sample (usually two to three grams) is extracted with cold water and then brought into a flask with 200 cc. water and 20cc. of twenty-five per cent. hydrochloric acid. Close the flask with a stopper bearing a reflux condenser and heat in the water bath for three hours. Filter and wash. Nearly neutralize the solution with caustic soda and make to definite volume. Determine dextrose by boiling twenty-five cc. with excess of Fehling's solution, using Allihn's table. Dextrose ($C_6H_{12}O_6$) $\times$ 0.9 equals starch ($C_6H_{10}O_5$). It is evident that pentosans and the insoluble glucosides, all of which yield reducing substances on hydrolysis, will tend to vitiate the results whenever present.

(b) *Maercker's Method*.—Heat the sample (previously extracted with water) with fifty cc. water to 90° to gelatinize the starch. Cool to 55° , add malt extract and maintain at 60° to 65° for thirty minutes, and then add ten cc. of one per cent. tartaric acid, close in a pressure flask and heat at 135° to 140° (three to four atmospheres pressures) for thirty minutes. Cool and treat again with malt extract as before. Filter, wash, add one-tenth the volume of twenty-five per cent. hydrochloric acid, and invert the solution according to Sachsse's method, or by boiling thirty minutes on the sand bath. Determine glucose as under Sachsse's method.

Maercker's method has been quite largely used in Germany and to a less extent in this country. I find no information in

regard to the effect of high temperatures and pressure on the solubility of the pentosans. My own results indicate that they are less readily affected by this treatment than might be supposed.

(b) *Diastase Method*.—Reinke seems to have been the first to discard the use of pressure and tartaric when working with diastase or malt extract. The sample, previous freed from water-soluble matter, is heated to boiling with water to completely gelatinize the starch, then cooled to 62°, malt extract added, and the whole kept at about this temperature till a drop no longer shows the starch reaction with iodine. Then filter, wash and invert the solution as described under Maercker's method. When working with samples containing but little fiber, it is well to add a little malt extract before heating as suggested by Hibbard.

The solution will filter more readily if the treatment with malt extract be continued about an hour. Even then the filtration may sometimes be very difficult, the filter quickly becoming clogged if suction is applied. In such cases I have found it convenient to cool the solutions, make to definite volume, filter through a dry plated paper, and invert fifty cc. of the filtrate in a 100 cc. flask.

Since diastase does not invert glucosides nor pentosans it follows that a substance to interfere with this method must be insoluble in cold water, but soluble in water at 65°, and must yield a reducing substance when treated with hydrochloric acid. An examination of the available literature failed to show any such constituent occurring in considerable quantity in grains or common grasses.

(c) *Guichard's Method*.—Boil the sample for fifteen minutes with 100 cc. saturated oxalic acid. Filter and wash. To the solution add one-tenth its volume of nitric acid (36° B.) and heat in the water bath for one hour. Clarify with bone black and polarize, calculating the readings to glucose. In obtaining the results given in the table, I substituted heating in the water bath for actual boiling with oxalic acid and estimated the final solution by Fehling's solution as usual, in order that the results might be strictly comparable with those obtained by the other methods.

(d) *Baudry's Method*.—Take a normal weight of the sample (2.688 grams for the Laurent polariscope), boil with eighty to ninety cc. water and four-tenths to five-tenths gram salicylic acid for forty minutes. Add water to nearly 200 cc., cool, add a few drops of ammonia, and fill to 200 cc. Filter and polarize in a 400 mm. tube. A modification consists in heating one hour in the water-bath instead of boiling. The rotation on which the above calculation is based, is $[\alpha]_D = 200.25^\circ$.

As a rule I was unable to get solutions sufficiently clear to polarize. Of course the ordinary clarifying agents cannot be used. The two samples containing the largest amounts of pentosans were treated as above for one hour in the water-bath, filtered, and the solution inverted with hydrochloric acid. The results are shown in the column headed "Baudry's Method Modified." Although the treatment appears quite mild, the results are both considerably too high.

(d) *Salicylic Acid Method*.—Weigh out one to three grams of the finely ground material, extract water soluble matter, and then bring into a flask with 100 cc. water and five-tenths gram salicylic acid. Place in the water-bath, heat to boiling, and keep in the boiling water till all the starch is rendered soluble (fifteen minutes), then filter quickly through muslin with suction and wash with hot water, or add water, cool, make to definite volume, filter through folded paper, and take an aliquot part of the solution. To the filtrate add one-tenth its volume of twenty-five per cent. hydrochloric acid and boil forty-five minutes on the sand-bath with reflux condenser and estimate glucose as usual.

To obtain the most accurate results, the time required to render all the starch soluble should be previously determined in each case. Five to ten minutes is usually sufficient. The residue after washing should be proved free of starch.

This method is the result of experiments on Baudry's process, but has only the solvent in common with the latter. Its chief interest lies in the fact that it gives fairly accurate results, even on the most difficult materials, without the use of diastase or other ferment. It is based on the observation that one-half per cent. salicylic acid, at the temperature of boiling water, renders

all starch soluble (capable of being filtered) before the pentosans are sensibly attacked.

Tabulation of Results.

The results obtained by the different methods are shown in Table I. Table II shows the results of qualitative tests for pentoses in the final solutions, from which the figures in the first table are obtained. The figures in 1, 2 and 3 are used to indicate the relative intensity of the positive tests.

TABLE I. PERCENTAGE OF STARCH BY DIFFERENT METHODS.

Sample.	Malt method.	Maercker's method.	Salicylic acid method.	Sachsse's method.	Baudry's method modified.	Guichard method.
Pure starch...	82.49	82.39	82.33	82.30	82.33	82.50
Wheat flour...	66.55	67.15	66.84	68.35		
Oatmeal	56.23	56.16	56.00	59.01		
Graham flour.	55.32		55.66	58.63		
Wheat bran...	20.97	21.07	21.57	38.82	29.74	31.36
Wheat straw..	4.39	4.80	4.41	22.69	5.33	8.93
Corn fodder...	0.96		0.92	20.13		

TABLE II. QUALITATIVE TESTS FOR PENTOSES IN SOLUTION.

Sample.	Cold water.	Malt method.	Maercker's method.	Salicylic acid.	Sachsse.	Baudry.	Guichard.
Wheat flour...	1	0	0	0	?	..	..
Graham flour..	?	0	..	0	1	..	..
Wheat bran ...	0	0	?	1	3	2	2
Wheat straw ..	0	0	0	0	3	?	2
Corn fodder...	0	0	..	0	3	..	..

The above results indicate that :

1. The diastase or malt method, properly executed, gives satisfactory results and cannot be vitiated by the presence of any substance now known to occur in considerable quantity in cereals or the common grasses.

2. The results shown by Maercker's and the salicylic acid methods are, in the main, fairly comparable with those of the diastase method, and the final solutions contained, with a single exception, only questionable traces of pentoses or none at all.

3. No method based on the chemical transformation of starch by acids before removal from other substances present can be accepted as generally applicable to the above classes of materials.

4. A qualitative test for pentoses in the final solution serves as a valuable check in working with any of the inversion methods.

PRINCIPAL METHODS PROPOSED FOR THE ESTIMATION OF STARCH.

A. Inversion Methods:

CHITTENDEN, Studies from the Lab. of Physiol. Chem., Yale Univ.; J. Anal. Chem., **2**, (1888), p. 153. Starch is dissolved by neutralized saliva, maltose and dextrin being formed. These are converted to glucose by hydrochloric acid.

EFFRONT, Bul. Soc. Chim., **47**, 5; J. Anal. Chem., **1**, 430. Starch is treated with diastase and the resulting maltose and dextrin determined as such.

GUICHARD, Bul. Soc. Chem., **7** (Ser. 3), 554. Dissolve by heating with saturated oxalic acid. Invert with nitric acid. Polarize the glucose solution and calculate to starch. See Stone, Bul. 43, U. S. Dept. Agr., Div. Chem., p. 162.

LINTNER, Ztschr. angew. Chem., 1888, **1**, 65. Starch is rendered soluble by heating under pressure with water alone. Filter through glass wool and invert solution by hydrochloric acid.

MAERCKER, Landw. Versuch-Sta., **25**, 107, used this method in his earlier analysis. It is sometimes called Maercker's Earlier Method. See Winton, J. Anal. Chem., **2**, (1888), 155.

MAERCKER, Ztschr. anal. Chem., **24**, 617; Chem. Ztg., **9**, 319; J. Anal. Chem., **2**, (1888), 156. Starch is dissolved by diastase, heated thirty minutes under pressure with dilute tartaric acid, filtered and inverted as usual. See Milkowski: Ztschr. Anal. Chem., **29**, 134. Winton, J. Anal. Chem., **2**, 156.

REINKE, Ztschr. anal. Chem., **29**, 472, omits the use of pressure and tartaric acid. See Stone, Bul. 43, U. S. Dept. Agr., Div. Chem., p. 166; J. Am. Chem. Soc., **16**, 726.

HIBBARD, J. Am. Chem. Soc., **17**, 64, adds malt infusion before gelatinizing to prevent balling. In inversion, boils on sand-bath with hydrochloric acid instead of heating in water-bath.

O'SULLIVAN, J. Chem. Soc., **45**, 1. Soluble carbohydrates, fats and albuminoids are first removed and the material then treated with diastase. Starch is calculated from the reducing power and opticity of the solution. The specific gravity serves as a check.

REMPEL, Ber. d. Chem. Ges., **18**, 621, dissolves by heating under pressure with dilute tartaric acid.

REINKE, Ztschr. anal. Chem., **29**, 472, substitutes lactic acid.

SACHSSE, Chem. Centrbl., 1877, 732, inverts with hydrochloric acid directly. See Lintner and Düll, Ztschr. angew. Chem., 1891, 537, Analyst, 1891, 216; Winton, J. Anal. Chem., **2**, 153; Stone, Bul. 43, U. S. Dept. Agr., Div. Chem., p. 162; Hibbard, J. Am. Chem. Soc., **17**, 64.

HORN, Chem. Tech. Anal. Org. Stoffe, heats under pressure before inversion.

DRAGENDORFF, *Plant Analysis*, p. 84, removes other substances liable to interfere by treating with alcoholic caustic potash, then inverts with hydrochloric acid.

B. Polarization of Soluble Starch :

BAUDRY, *Rev. de Chim. Ind.*, **5**, 81; *Ztschr. Spiritus Ind.*, **15**, 41; *Chem. Centrbl.*, 1892, **I**, 339, 509; *Agl. Sci.*, **7**, 186. Dissolve by heating with salicylic acid or zinc chloride, filter and polarize. $[a]_D^{20} = 200.25^\circ$. See Deltour, *Bul. Ass. Chim. Belg.*, **6**, 154; *Chem. Ztg. Rep.*, **17**, 42; Stone, *Bul. 43*, U. S. Dept. Agr., *Div. Chem.*, p. 162; *J. Am. Chem. Soc.*, **16**, 726.

OST, *Chem. Ztg.*, **19**, (1895), 1501. *Analyst*, **20**, 235, 226. Solution obtained by heating with water under pressure. $[a]_D = 196.7^\circ$.

C. Precipitation of Soluble Starch by Alcohol.

HONIG, *Chem. Ztg.*, **14**, 868, 902; *J. Anal. Chem.*, **5**, 234. Sample is heated with glycerine, cooled, starch precipitated by alcohol and ether, and subsequently removed by hydrochloric acid from the insoluble fiber (cellulose).

LECLERC, *J. de Pharm.*, **21**, 641; *Bul. 31*, U. S. Dep't. Agr., *Div. Chem.*, p. 62. Dissolve by zinc chloride at 108° , precipitate by alcohol, filter and weigh.

D. Precipitation of Starch-paste by Baryta.

VON ASBOTH, *Rep. anal. Chem.*, No. 20; *Chem. Ztg.*, **13**, No. 37, 591; *Analyst*, **12**, 138; *J. Anal. Chem.*, **2**, 152. See Spence, *J. Soc. Chem. Ind.*, **7**, 77; *J. Anal. Chem.*, **3**, 22; Seyfert, *Ztschr. angew. Chem.*, No. 5, (1888), 126; Milkowski, *Ztschr. anal. Chem.*, **29**, 134; Winton, *J. Anal. Chem.*, **2**, 162; Stone, *Bul. 43*, U. S. Dept. Agr., *Div. Chem.*, p. 162; Monheim, *Chem Centrbl.*, 1888, 425.

VULTE, *Trans. of N. Y. Academy of Sciences*, Oct., 1890. Describes the details most efficient in preventing the absorption of carbon dioxide from air and shows the calculation of the factors used.

E. Loss of Weight :

ALLEN, *Com. Org. Anal.*, Vol. I, second edition, p. 344. Starch is calculated from increase of specific gravity of the malt extract on solution of starch in same.

KRUGER, *Fort. Chem. Nahrung.*, **9**, 567, *Chem. Centrbl.*, 1895, **1**, 1085. Weigh out two samples. Wash one with solution of sulphur dioxide. Treat the other with malt extract. Difference in weight equals starch.

F. Determination with Iodine :

DEMISTADT and VOIGTLANDER, *Chem. Centrbl.*, (1894) **2**, 322, colorimetric method; SEYFERT, *Ber.* **21**, Ref. 298, gravimetric method.

G. Calculation from Specific Gravity:

- a.* Tables and formulae for potatoes. Behrend, Maercker and Morgan, Agric-Chem. Centrbl., 1880, 452; Horn, Chem. Tech. Anal., 64. Heidespreim, J. Chem. Soc., 32, 233. Pohl, Weiner Academie-Berichte, 8, (1852), 42. Post, Chem. Tech. Anal., 861.
- b.* Formula for commercial starch. Saare, J. Soc. Chem. Ind., 3, 527. Allen, Com. Org. Anal., Vol. I (2nd), p. 346.
- c.* Apparatus for measuring water displaced. Girard, Aime and Flourent, Centrbl. Agric-Chem., 21, 858.

2. *The Determination of True Pentosans.*—Reference has been made to the interpretation of furfural as a measure of pentosans (p. 17). It has been shown that by hydrolysis of the hemicellulose solution, pentoses only are obtained. Since, moreover, isolated pentosans are readily dissolved by dilute acids, it is evident that hydrolysis of this solution and determination of the pentosans formed must give a fairly accurate measure of the true pentosans. The kind and strength of acid used should be a subject for further study. Sulphuric acid was used in this work because the pentoses are so little altered by long boiling with it.¹ The reducing power was referred to dextrose by Allihn's table and the pentose taken as ninety-seven per cent.² of the amount of dextrose indicated. $\text{Pentose} \times 0.88 = \text{pentosan}$.

3. *The Determination of Cellulose.*—The three methods compared have been described above (pp. 10, 11 and 12). Exposing to chlorine after simple wetting without alkaline treatment, and then proceeding as in the chlorination method has been tried and the results are tabulated with those obtained by the regular methods. The fiber itself is also shown for purposes of comparison.

Method.	Description of Product.			
	Yield per cent.	Furfural per cent.	Nitrogen per cent. fixed	Ferricyanide per cent. Color.
Cross and Bevan	66.5	5.62	0.00	0.92 Light cream.
Lange	41.2	3.96	0.03	0.89 Nearly white.
Schulze	66.0	7.00	0.22 ³	6.04 Light brown.
Chlorination alone....	84.3	8.89	0.08	2.02 Very light brown.
Original sample		11.46	0.25	11.17 Brown.

¹ Tollens and Schulze: *Landw. Versuch-sta.*, 40, 379-384.

² Stone: *Am. Chem. J.*, 13, 73.

³ Exclusive of 0.07 per cent. ammoniacal nitrogen (from the wash solution) determined by distillation with magnesia.

It is evident that no one feature can be urged as a criterion in judging between the methods but all must be taken into consideration. Such a comparison shows the superiority of the chlorination method. It also suggests the possibility of increasing the yield without detriment to the quality of the product, by making the preceding alkali treatment less severe.

The reaction with the ferricyanide solution (p. 8) seems of considerable value in testing the purity of celluloses. When the sample increases only slightly (about one per cent. or less) in weight and is dyed only a bluish-green instead of a deep blue color, it is at least a very strong indication that lignin substances are not present in notable quantity. When the original fiber is dark-colored, the appearance of the cellulose will also serve as a guide, for unless this color has disappeared, it is probable that the lignin group has not been completely removed.

The significance of the furfural-yielding property of these celluloses has been discussed in the first part of this paper.

4. *Lignin and Allied Substances.*—These are determined by loss of weight when cellulose is isolated from the fiber. The general characteristic feature of the substances is their susceptibility to alkali. This is the principle upon which Lange's method is based; but since the treatment called for by the latter evidently attacks the cellulose, it is best to remove the more readily soluble portion by boiling with dilute alkali and then treat with chlorine to convert the remainder into a more easily soluble compound. The first step is also introduced in the current analytical methods, and the term lignin is usually restricted to the portion which remains with the cellulose in the "crude fiber." There is, however, no established chemical difference on which to rest such a distinction, and it is, therefore, omitted here, although the step might easily be introduced if desired.

B. DETERMINATION OF COPPER REDUCTION.

Wherever in this paper, the reducing power of a solution is given, the determination has been made by adding twenty-five cc. of the solution to be tested to an excess of boiling Fehling's solution under the usual conditions of dilution, boiling exactly two minutes, collecting and estimating the cuprous oxide and taking the corresponding value for dextrose from Allihn's table.

In most cases the cuprous oxide was determined as follows:¹ Collect the reduced oxide on asbestos in a filtering tube and wash well with water. Dissolve on the filter with nitric acid and wash thoroughly into a flask. To the solution add excess of sodium carbonate and dissolve the precipitate with one cc. of ammonia (0.96 sp. gr.). Titrate with a recently standardized solution of potassium cyanide until the color disappears.

The method is rapid and accurate since no other heavy metals than copper can be present. Before adopting it I checked it against the electrolytic method and made a large number of experiments to show that varying amounts of sodium nitrate had no effect upon the result. After a little practice in determining the end reaction, I believe the results obtained should be as accurate as by the gravimetric methods, and much more accurate than simple titration with Fehling's solution.

C. DETERMINATION OF FURFURAL.

The method used was essentially that of the Association of Official Agricultural Chemists, but as that may not be available to all, and as some of the details were modified, it is given in full.

From two to five grams of the sample are placed in a flask with 100 cc. of hydrochloric acid of 1.06 specific gravity. The mixture is distilled and the distillate collected in a graduated receiver. Each thirty cc. lost by distillation (which should require from ten to fifteen minutes), is replaced by thirty cc. of hydrochloric acid of the strength mentioned above. Continue the process until a drop of the distillate produces no red coloration in a slip of filter paper moistened with anilin acetate. Eight to twelve distillations are usually required. Add to the distillate sufficient water to bring to a volume of 400 cc. and for every fifty cc. of water added, add 10.2 grams sodium chloride. Neutralize exactly with sodium carbonate, and add ten cc. of a solution of phenylhydrazine acetate, made by mixing twelve grams phenylhydrazine with seven and five-tenths grams glacial acetic acid and diluting to 100 cc. Stir thirty minutes and let stand in a dark closet until the following day. Filter on glass wool in glass filtering tubes six to seven inches long, removing the pre-

¹ Lavene: *School of Mines Quarterly*, 17, 470.

precipitate which adheres to the beaker by means of a feather. Dry to constant weight in an air-bath at 55° to 60° in a slow current of dry air. A partial vacuum is produced in the tube by means of suction, the supply of air being regulated by stop-cocks. Finally the precipitate is dissolved by hot alcohol, the tube dried and weighed and the loss of weight calculated as furfural hydrazone. For reducing to furfural and to pentosans the following formulas were used :

$$\begin{aligned}\text{Hydrazone} \times 0.516 + 0.0104 &= \text{furfural,} \\ \text{Furfural} \times 1.84 &= \text{pentosans.}\end{aligned}$$

The factor for pentosans is obtained by averaging those of xylan and araban.

The following results obtained after only a very few preliminary trials, show what agreement may be expected without much practice.

Sample.	Weight taken. Grams.	Hydrazone obtained. Grams.	Furfural. Per cent.
A	{ 3.860	0.836	11.46
	{ 3.860	0.829	11.36
	{ 3.860	0.845	11.57
B	{ 3.870	0.393	5.54
	{ 3.870	0.404	5.70
	{ 3.670	0.559	8.14
C	{ 3.670	0.561	8.17
	{ 3.670	0.551	8.03
	{ 3.628	0.314	4.78
D	{ 3.628	0.311	4.74

PART III. PHYSIOLOGICAL.

INTRODUCTORY.

In questions of nutrition the proportion of food and of each of its proximate ingredients which is digested, *i. e.*, which leaves the alimentary canal and enters the physiological interior of the body, is evidently the basis of all further studies. While the different substances digested are not all utilized to the same extent, and while the undigested residue undoubtedly performs an important physiological function, these facts only serve to show the necessity of further study based upon the "digestive coefficients" and do not detract from the value of the latter when properly interpreted. Such digestive coefficients to be comparable must of course be determined by uniform methods. The analytical method used by the experiment stations in this

country is published by the U. S. Department of Agriculture.¹ The work of these stations, as well as important foreign work, is periodically reviewed in the Experiment Station Record.² A yearly digest of the subject is included in the *Jahresbericht für Agricultur-chemie*, and it has been treated by Dietrich and Konig.³

It is mainly upon these experiments with animals that we are obliged to base our estimates of the digestibility of wheat and wheat products since the published results of experiments with men⁴ are very incomplete. But the results obtained by current methods of analysis give little information regarding the carbohydrates, this group being simply divided by an arbitrary line (sometimes considered a rough imitation of the digestive process, into "nitrogen-free extract" and "crude fiber."

Recently a number of determinations of digestibilities of total furfural-yielding substance have been reported, especially by Stone⁵ and Lindsey.⁶ Those of wheat bran fed to different animals (sheep and rabbits) and at different times vary from fifty-nine to sixty-four per cent.

DIGESTION EXPERIMENT.

Having studied the chemical nature of the principal carbohydrates of wheat it is interesting to find the digestibility of each as determined by an actual experiment. I was fortunate in obtaining, through the kindness of Mr. H. J. Patterson, of the Maryland Experiment Station, samples of the food and feces from an experiment conducted by him⁷ upon a steer fed with wheat bran only. The total dry matter digested was 68.33 per cent. of the whole. The samples were analyzed by the method outlined above, the small amount of ash in the cellulose being neglected. The results, calculated to the dry basis, are as follows:

¹ Bul. 46, Division of Chemistry.

² Published by the U. S. Department of Agriculture.

³ Zusammensetzung und Verandlichkeit der Futtermittel.

⁴ Compiled by Atwater: Bul. 21, Office of Experiment Stations, U. S. Department of Agriculture.

⁵ *Am. Chem. J.*, 14, 9; *Agr. Sci.*, 5, 6.

⁶ Mass. Station Report for 1894, p. 185-186.

⁷ Maryland Station, Bul. 41, pp. 137-138.

	Food. Per cent.	Feces. Per cent.	Per cent. Digested.
Soluble carbohydrates as dextrin	7.2	0.7	96.9
Starch	17.7	0.0	100.0
Free pentosans	17.5	18.7	66.2
Cellulose	8.5	20.2	24.8
Lignin and allied substances	11.6	23.2	36.7
Protein ¹	20.49	11.04	82.96
Ether extract ¹	6.92	12.52	42.73
Ash ¹	6.05	11.04	42.21
Undetermined	4.04	2.60	...
Nitrogen-free extract ¹	55.59	41.93	76.08
Crude fiber ¹	10.96	23.47	32.21

We see from the above that the digestibilities of the insoluble carbohydrates range from 25 to 100 per cent. The "nitrogen-free extract" with a mean digestibility of seventy-six per cent. is composed of substances whose percentage digestibilities vary from 100 to less than forty. Even in the case of the crude fiber the digestibility of one of the constituents considerably exceeds that of the other. It must be borne in mind in this connection that the group of lignin and similar substances is divided by the current methods, part being included in the nitrogen-free extract and part in the crude fiber.

DIGESTIBILITY AND PHYSIOLOGICAL VALUE.

The final utilization of the normal carbohydrates absorbed by the healthy animal body is known to be practically complete. With lignin and pentosans or pentoses, the case seems to be different. It is held² that the lignin of vegetable tissues supplies directly the benzol radicle of the hippuric acid of the urine of herbivorous animals and is, therefore, but little oxidized in the body.

In the case of pentoses and pentosans whose identification is easy, the experimental methods are more direct, yet the results are very conflicting.

Ebstein³ found that either arabinose or xylose given to men in doses of twenty-five grams, quickly appeared in the urine

¹ From Patterson's analysis.

² Meissner and Shepard: Untersuchungen über das Entstehen der Hippursäure im thierischen Organismus. Hanover, 1866. Stutzer: *Ber. d. chem. Ges.*, 8, 575. Weiske: *Zeit. Biol.*, 12, 24.

³ *Archiv. pathol. Anat.*, 129, 401; *Centrbl. med. Wissens.*, 1892, 577.

unchanged, and he concluded that these sugars were without nutritive value, or at least nearly so. This result has been largely quoted and its importance perhaps overestimated. Salkowski,¹ and Cramer,² in numerous independent experiments upon men, rabbits, and fowls, found that these sugars were mainly utilized, only about one-fifth (according to Salkowski) being given off in the urine. In many cases (according to Cramer) it was shown by killing the animals that the feeding of pentoses had caused an increased storage of glycogen in the liver. Finally Fretzel,³ experimenting upon rabbits with xylose, has concluded that this sugar does not tend, either directly or indirectly, to promote the storage of glycogen.

The advisability of distinguishing between starch and pentosans in food analyses is thus emphasized not only by the difference in their digestibility but also by the possible difference in physiological value of the digested portions.

SUMMARY.

The chief results of this investigation may be briefly stated as follows :

1. As determined by the analysis of the osazones, only the pentoses, xylose and arabinose, result from the hydrolysis of the hemicellulose. This is, therefore, practically identical with the free or normal pentosans which the wheat contains.

2. The preparation of cellulose from the fiber insoluble in dilute acid was found to be best effected by means of alkali and chlorine as described by Cross and Bevan.

The dilute boiling alkali removes matter which appears to contain a condensed form of pentosan, since it yields furfural on distillation and gives a red color with phloroglucin reagent but does not yield any notable amount of reducing sugar on boiling with dilute sulphuric acid.

The lignin not removed by dilute alkali forms with chlorine an alcohol-soluble compound containing 26.7 per cent. of chlorine corresponding to the "lignone chloride," $C_{13}H_{18}Cl_4O_2$, of Cross and Bevan.

¹ *Centrbl. med. Wissens.*, 1893, 193.

² *Ztschr. Biol.*, 19, 484.

³ *Archiv. ges. Physiol.*, 55, 273.

3. The cellulose obtained as just mentioned, or by fusion of the fiber with strong alkali (Lange's method), contains furfurolyielding bodies whose deportment toward reagents indicates the presence of penta-anhydride, probably in combination with a part of the hexa-anhydride or normal cellulose.

When dissolved in sulphuric acid, diluted, and hydrolyzed, a small quantity of dextrose only was obtained as osazone.

4. The property of dyeing in a solution of ferric chloride and potassium ferricyanide is possessed in a marked degree by the wheat fiber, and the reaction has been found useful in testing the purity of "cellulose" residues. In this respect, as in the formation of the lignone chloride, the lignified tissue of wheat resembles that of jute, the typical lignocellulose.

5. No notable amount of oxycellulose has been found in any of the preparations from the wheat fiber.

6. The relations of the constituents under consideration may be represented as follows :

A. Starch granules.	} Separated anatomically.	
B. Outer seed-coat.		
a. Free pentosans.	} Associated in position but not chemically com-	
b. Lignified tissue.		bined.
ba. Cellulose.....	} Apparently in	} In chemical
baa. Hexa-anhydrides.		
bab. Penta-anhydrides.		
bb. Lignin	} combination.	

Undefined substances, apparently of a condensed nature, associated with and doubtless allied to the lignin.

7. The determination of starch has been carefully studied and the methods now available are quite satisfactory. An approximate separation of free pentosans, lignin and its allies, and cellulose, may be effected by means of the method proposed in this paper.

8. Thus determined, the digestibilities in a case where wheat bran had been fed alone were found to be : Starch, 100 per cent. ; free pentosans, 66.2 per cent. ; lignin and allied substances, 36.7 per cent. ; cellulose, 24.8 per cent.

9. From the analyses given in this paper and the best available results of other experimenters, the proportions present in normal mature wheat, air-dried, are calculated as follows :

Starch	54.0 to 59.0 per cent.		
Free pentosans.....	3.5 "	4.5 "	" "
Lignin and allied substances	2.0 "	2.5 "	" "
Cellulose	1.5 "	2.0 "	" "
Total	<u>61.0</u> "	<u>68.0</u> "	" "

